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13. ABSTRACT (Maximum 200 words)

This research explored the effect of cytokines and neurosteroids on the GABAergic and glutamatergic neurotransmitter systems in the CNS. Interleukin-1 (IL-1) augmented $GABA_A$ receptor function in behavioral, neurochemical and electrophysiological paradigms both *in vivo* and *in vitro*. These effects of IL-1 were inhibited by the IL-1 receptor antagonist suggesting an indirect effect of IL-1 at its own receptor in the modulation of the $GABA_A$ receptor. Subsequent experiments demonstrated a negative modulatory role of IL-1β on NMDA receptor-mediated intracellular calcium increases which was unique to both IL-1β and to the NMDA receptor. These results provided the first direct evidence of a functional interaction of IL-1β with the NMDA receptor and implies a beneficial role of this cytokine in neurodegenerative processes. Additional work in our laboratory confirmed that pregnenolone sulfate (PS) potentiated the NMDA receptor-mediated increases in calcium flux, most likely acting at a unique steroid recognition site on the NMDA receptor. Further studies demonstrated a neurotoxic effect of PS on these cortical neurons *in vitro*. This study also demonstrated a synergistic toxic effect of PS and NMDA/glycine, providing additional evidence of the possible involvement of PS in the excitotoxic damage.

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FINAL PROGRESS REPORT

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PRINCIPAL INVESTIGATORS: Dr. Lawrence G. Miller
Dr. Jeanne M. Fahey

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GRANT TITLE: IL-1 effects in brain (1 June 1991-31 May 1994)
Cytokine modulation of glutamatergic function in brain
(1 June 1994 - 31 December 1996)

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OBJECTIVE: To determine the effect of cytokines and neurosteroids on the major inhibitory and excitatory neurotransmitter systems in the CNS.

APPROACH: In initial studies, we determined the effect of interleukin-1 (IL-1) on $GABA_A$ receptor function both *in vitro* and *in vivo*. GABA-dependent chloride uptake was performed in mouse cortical synaptosomes. Additional studies were done in male CD-1 mice to determine the effect of IL-1 and related cytokines on open-field activity and pentylenetetrazol-induced seizures. Electrophysiological studies were performed on chick cortical neurons using whole-cell voltage clamp technique.

Primary chick cortical neurons were exposed to interleukin-1 (IL-1β) and interleukin-6 (IL-6) in the presence and absence of N-methyl-D-aspartate (NMDA) and glycine. A functional assay of NMDA-mediated calcium flux using Fura-2-AM, a cell permeant intracellular fluorescent calcium indicator, was used to assess glutamatergic function. Several receptor agonists and antagonists were employed to determine both IL-1β activity and receptor subtype specificity. Similar experiments were also conducted using the neurosteroid pregnenolone sulfate (PS). Compounds involved in the nitric oxide (NO) signal transduction system were employed to determine the mechanism of IL-1β and PS activity.

An *in vivo* study was also conducted to examine the effect of PS on open-field locomotor activity in male CD-1 mice. *In vitro* neurotoxicity studies were conducted with PS utilizing an [^{3}H]oubain binding assay to quantify cell loss. Primary chick cortical neurons were exposed to PS in the presence and absence of NMDA and glycine to determine the role of this neurosteroid in excitotoxicity and cell death. In addition, several collaborative studies were undertaken to investigate the role of cytokines and neurosteroids on novel *in vivo* models of neurodegenerative diseases.

ACCOMPLISHMENTS: IL-1 (100 pg.ml - 10 ng/ml) augmented $GABA_A$ receptor function in cortical synaptic preparations. This effect was inhibited by incubation with the specific IL-1 receptor antagonists (IL-1ra). The related cytokines tumor necrosis factor (TNF) and interleukin-6 (IL-6), had no effect on GABA-dependent chloride transport. An analog supplied by Dr. C. Dinarello was also ineffective. Finally, a rat IL-1 fragment supplied by Dr. J. Krueger was slightly, but not significantly, more effective than IL-1. Similar enhancement of $GABA_A$ function was observed in tissue prepared from mice previously injected intraperitoneally with IL-1 (1 μg). Electrophysiological studies in cultured primary cortical neurons demonstrated that IL-1 enhanced the GABA-mediated increase in chloride permeability, whereas IL-1 alone produced no alterations in

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resting conductance. Behavioral studies indicated that IL-1 is similarly active *in vivo*. Mice treated with IL-1 showed a decrease in open-field activity and an increase in pentylenetetrazol-induced seizures.

Extensive studies have been completed assessing the effects of IL-1 on glutamatergic receptor subtypes. These receptors appear to function in a manner complementary to $GABA_A$ receptors and the balance determines overall CNS activation. IL-1β, in the absence of NMDA and glycine, had no effect on intracellular calcium levels. All concentrations of IL-1β (0.01 pg/ml - 100 ng/ml) significantly attenuated the increase in intracellular calcium seen in the presence of NMDA/glycine (500 μM/50 μM) with and without the addition of spermine (250 μM) at all but the highest and lowest concentrations as well as 100 pg/ml IL-1β. The decreases in intracellular calcium produced by IL-1β in the presence of NMDA/glycine alone and in combination with spermine were antagonized by 10 ng/ml IL-1ra. The biologically similar cytokine IL-6 produced no changes in intracellular calcium in the presence of NMDA/glycine. IL-1β had no effect on the kainate-mediated increases in free calcium. In separate experiments, we examined the role of NO on the modulation of the NMDA receptor by IL-1β. N^g-Monomethyl-L-argine (L-NMMA), a nitric oxide synthase (NOS) inhibitor, had no effect at any tested concentration (10 μM - 500 μM) on the decrease in intracellular calcium caused by IL-1β in the presence of both NMDA/glycine and the endogenous polyamine spermine. L-arginine (10 μM), the precursor of NO, significantly increased intracellular calcium, antagonizing the effect of IL-1β.

The second area on which we focused concerned neurosteroid modulation of the glutamatergic system. PS, in the absence of NMDA/glycine, significantly elevated intracellular calcium at 250 μM and 500 μM. This increase in free calcium was significantly attenuated by the prior addition of CNQX, dizocilpine or nimodipine. In the presence of NMDA/glycine with and without the added polyamine spermine, both 50 μM and 100 μM PS produced a further significant rise in intracellular free calcium. The prior addition of CNQX, dizocilpine or both compounds together significantly inhibited these elevations in free calcium in both the presence and absence of spermine. Further experiments were conducted to determine the role of NO on neurosteroid modulation of the NMDA receptor. L-NMMA (10 μM - 100 μM) had no effect on the increase in intracellular calcium seen in the presence of PS and NMDA/glycine with and without spermine. L-arginine (100 μM), however, antagonized the rise in free calcium seen in the presence of NMDA/glycine in the absence of spermine only.

We next expanded our previous finding that PS, in the absence of NMDA/glycine, significantly elevated intracellular calcium at 250 μM and 500 μM. This increase in free calcium indicated a possible neurotoxic effect and, therefore, experiments were conducted to examine the effect of PS on neuronal cell death alone and in combination with NMDA/glycine. Neuronal survival was significantly decreased in the presence of PS at 24 hr (500 μM), 48 hr (500 μM) and 72 hr (250 μM and 500 μM) relative to controls. In a second study, co-application of non-toxic concentrations of NMDA/glycine (500 μM /50 μM) and PS (250 μM) for 48 hr significantly reduced neuronal survival in these neurons relative to either compound alone.

To determine the behavioral effect of PS, male CD-1 mice were tested for open field locomotion after the administration of various doses (10-150 mg/kg) of this neurosteroid. PS at doses of $\geq$ 50 mg/kg decreased the initial high level activity that was seen in the first 10 minutes of testing and reduced the overall activity of the mice in the

entire test period. The lowest dose of 10 mg/kg PS significantly increased distance traveled in the first 10 minute interval. Flumazenil, a $GABA_A$ antagonist, and CPP, an NMDA antagonist, had no effect on the distance traveled by the 50 mg/kg PS group in the first 10 minute period. Nimodipine, an L-type VSCC antagonist, increased distance traveled in the initial 10 minute period. PS administered with nimodipine inhibited the increase in activity seen with the calcium antagonist in the first 10 minutes.

The final area on which we have focused concerns neurosteroid and cytokine effects on animal models of neurodegenerative diseases. As stated above, we have demonstrated a neurotoxic effect of PS on cortical neurons as well as a synergistic effect of this neurosteroid in the presence of a non-toxic concentration of NMDA. Prior to this, our laboratory has demonstrated that IL-1β has a negative modulatory effect on NMDA receptor-mediated intracellular calcium increases in primary chick cortical neurons which appears to be unique to both IL-1β and to the NMDA receptor. We have now undertaken two collaborative studies to examine the effect of IL-1β and PS on animal models of diseases whiich appear to be mediated, to some extent, by the NMDA receptor.

Jeanne Ryan and colleagues (1990, 1993) have developed a rat model of wandering in Alzheimer's disease using intrahippocampal injections of the neurotoxin colchicine. In collaboration with her laboratory at SUNY Plattsburgh, we are presently examining the effects of both IL-1β and PS on two behavioral components of this model which may contribute to wandering in Alzheimer patients: spatial memory impairments (Morris Water Maze) and psychomotor dysfunction (spontaneous alteration in the T-maze).

Previously, Fahey and Isaacson (1989) had developed an acute model of global ischemic damage using systemic sodium nitrite administration. More recently, our laboratory, in collaboration with Robert Isaacson (SUNY Binghamton), has expanded that model to allow histological determination of cellular changes up to 60 days following a single injection of sodium nitrite. Histological damage is assessed using several stains: cresyl violet, GFAP, PTAH and the Bielschowsky silver stain. In collaboration with his laboratory, we are presently examining the effects of both IL-1 and PS on this histological model of global ischemia.

SIGNIFICANCE: Although the mechanism for IL-1 effects on GABAergic transmission remains uncertain, our results support a specific interaction with the IL-1 receptor. This finding is based on the antagonism of IL-1 effects by the receptor antagonist as well as the lack of effect of IL-6 and TNF. In addition, the lack of change in GABA-dependent chloride uptake in preparations exposed to the IL-1 receptor antagonist alone may indicate that endogenous IL-1 has little effect on the $GABA_A$ receptor in the basal state. Effects of IL-1 on GABAergic function suggest that, during infection, sepsis or injury, IL-1 might enhance host adaptation by promoting inhibition in the CNS. This effect of IL-1 could be protective against associated events with potential excitatory effects, such as fever and electrolyte alterations.

Our results support a negative modulatory role of IL-1β on NMDA receptor-mediated intracellular calcium increases which appears to be unique to both IL-1β and to the NMDA receptor. These experiments provide the first direct evidence of a functional interaction of IL-1β with the NMDA receptor and implies a beneficial role of this cytokine in neurodegenerative processes thought to be mediated via the glutamatergic neurotransmitter system.

The present work also confirms that PS potentiates the NMDA

receptor-mediated increases of calcium flux, most likely acting at a unique steroid recognition site on the NMDA receptor. While the mechanism of action of PS is still not completely known, accumulating evidence from this laboratory and others points to the involvement of PS in excitatory neurotransmission and possibly in excitotoxicity and cell death which is largely mediated by the NMDA receptor.

The signal transduction experiments provide the first insight into the possible mechanism of action of the modulatory effects of IL-1β and PS on the NMDA receptor. These data suggest a role for the NO signal transduction system in the modulation of the NMDA receptor by both IL-1β and PS, providing evidence of NO as the second messenger system linking both of these compounds and NMDA receptor in the CNS.

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